

**Simulating Gene Expression Patterns of
Neurogenesis, Somitogenesis, and Morphogenesis
During Zebrafish Embryo Development**

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Abstract

During embryo development, it is essential that relatively homogenous groups of cells undergo differentiation to form spatially different patterns and eventually take on many different functions. Intercellular communication and morphogen gradients are two aspects that have been shown to play roles in determining cell fate. To understand better how these activities result in pattern formation, we utilized the NetLogo programming environment to simulate these processes. Using this program, we were able to observe visually the possible pattern formation of gene expression from activation and inhibition of genes, intercellular interactions, and the exposure to morphogen gradients. The three developmental phenomena of zebrafish embryos we studied were neurogenesis, somitogenesis, and morphogenesis during anterior/posterior patterning. The model for neurogenesis examines how autocatalysis and lateral inhibition (notch signaling) are required to form stable patterns. In addition, we investigated to what extent the geometry of the domains and the initial noise in the *her* gene expression help determine a cell's fate. To study somitogenesis, we explored how transcription and translation delays coupled with notch pathway and independent moving wave-front activity of *fgf* may contribute to the oscillation and synchronization of gene expression during formation of somites. Lastly, we used our models to examine how time and concentration of a morphogen gradient may signal gene activation and eventually form patterns of cells with stable and differing gene expression. Although, there is much room to study in more detail the systems of equations and the numerical analysis we used, overall this method is a good addition to the traditional methods of studying how gene-expression patterns may develop.

Introduction

Zebrafish as a model organism has become popular for studying developmental biology for many reasons: zebrafish are vertebrates, they are inexpensive and easy to maintain and breed, they are experimentally accessible, and are transparent during early development. The ease with which the embryo's DNA can be manipulated and the short period of embryogenesis make them an ideal system for studying different phenomena during development. Furthermore, there is a large community of resources for zebrafish information (<http://www.zfin.org>).

In the process of going from a uniform mass of cells to cells with differing gene expressions and function, communication between cells and exposure to different cellular environments are vital in determination of a cell's fate. We studied the role of these actions in neurogenesis, somitogenesis, and morphogenesis for anterior/posterior patterning during zebrafish embryo development.

Neurogenesis

In early zebrafish development, the neural plate forms domains containing cells that eventually become neurons. It has been shown that *neurogenin1* (*ngn1*) is a proneural gene that is expressed in these domains [1-9]. However, the number of cells within these proneural domains that become neurons is only a fraction of the total number of cells. Lateral inhibition coupled with autocatalysis is believed to play a key role in the cell's fate within the clusters of cells, see Fig. 1. Autocatalysis alone is not sufficient to differentiate cells within a neighborhood; if this was the case, all cells that express *ngn1*, no matter how small initially, would eventually become neurons. Lateral inhibition, a competitive process where one cell's autocatalysis process inhibits the same process in the neighboring cells, is necessary to prevent the over production of neurons. The Delta/Notch signaling provides the lateral inhibition that prevents neurogenesis in the adjacent cells by promoting *hairy* (*her4*) gene, which in turn inhibits the production of *ngn1*.

Somitogenesis

In many vertebrates, somites are formed synchronously on both sides of the body axis from a group of cells that buds off sequentially at the anterior end of the unsegmented presomitic mesoderm (PSM) [10-13]. Somites give rise to skeletal muscles, vertebrae, and some dermis. An essential part of this periodic process is the segmentation clock, the oscillator that produces the cyclic expression of specific genes. There have been many theoretical models developed to explain the mechanisms of this oscillator: cell cycle model [14], clock and induction model [15], clock and trail model [16], and the oscillator and traveling waves [16-24, 29]. Of all the references cited, only the cell cycle model [14] and Lewis's oscillator model [29] provided mathematical analysis of their theoretical model.

There have been findings that support that the notch signaling plays a critical role [20], see Fig. 2, and that *fgf* is important in determining somite boundary position [23]. Furthermore, the target genes *her1*, *her7*, and *deltaC* expressions were found to oscillate during somitogenesis. However, *deltaC* alone cannot oscillate. It requires the oscillation of *her1* and *her7*, thus *her1* and *her7* are excellent candidates for the central mechanism of the oscillator [24].

Anterior/Posterior Morphogenesis

The morphogen Wnt, acting through β -catenin and its antagonists secreted by the vertebrate organizer, regulate the compartmentalization of gene expression along the rostral-caudal axis during the development of the vertebrate nervous system. Wnt signaling, regulated by β -catenin, is responsible for posteriorizing activities that facilitate the expression of caudalizing genes. The ectopic expression of *wnt* has led to a loss of rostral neural domains and expansion of caudal domains, while ectopic suppression of *wnt* signaling has resulted in the opposite. The study of zebrafish headless (*hdl*) mutants reveals that the loss of head phenotype is due to mutation in the *tcf3* gene, a member of the Tcf/Lef family. In *hdl* mutants, the hindbrain and spinal cord are relatively unaffected, but there is an increase in the size of the midbrain-hindbrain boundary (MHB) and a loss of forebrain. This is consistent with *tcf3* as the repressor of caudal target genes. Furthermore, *tcf3b*, a homolog of *tcf3*, has been shown to limit caudalization activity of *hdl* mutants [25-26].

Methods

NetLogo

NetLogo is the next generation in multi-agent modeling languages that started with StarLogo. Daniel Bobrow and Wallace Feurzeig at Bolt, Beranek and Newman, Inc., and Seymour Papert, at MIT in the 1960's, originally developed StarLogo. In 2002, a team at Northwestern University led by Uri Wilensky and Seth Tisue developed NetLogo. As with StarLogo, it has a programmable environment ideal for studying the emergence properties of complex systems developing over time. This environment utilizes Logo language, which supports agents and parallelism. Three different types of agents make up the NetLogo world: turtles, patches, and observers. The world is divided into grids called patches, the turtles are agents that can move about the patches, and the observer is looking at the world. All three agents may have unlimited variables and can carry out activities simultaneously. As one can see, this environment is ideal to study the world of zebrafish embryogenesis. Information and programs may be downloaded from <http://ccl.northwestern.edu/netlogo>.

Models

Neurogenesis

Our lateral inhibition model is based on Meinhardt's model for self-enhancement (autocatalysis) and long-range inhibition [27-28], the nonlinear system of differential equations

$$\frac{\partial A_{ngn}}{\partial t} = P_{ngn} \left(\frac{A_{ngn}^2}{(A_{her} + 1)(1 + S_{ngn} A_{ngn}^2)} \right) - R_{ngn} A_{ngn} = f_1, \quad (1)$$

$$\frac{\partial A_{del}}{\partial t} = P_{del} \left(\frac{A_{ngn}}{1 + S_{del} A_{del}} \right) - R_{del} A_{del} = f_2, \text{ and} \quad (2)$$

$$\frac{\partial A_{her}}{\partial t} = P_{her} C_{noich} A_{tot_del} - R_{her} A_{her} = f_3. \quad (3)$$

The A variables are the amount of expression. The parameters P and R are the rates of production and the rates of degradation, respectively. The subscripts *ngn*, *del*, and *her* represent genes *ngn*, *delta*, and *her*. The parameter S_{ngn} is the rate of saturation of *ngn*, and S_{del} is the rate of self-inhibition of *delta*. The variable A_{tot_del} is the total *delta* in the neighboring cells. The

constant, C_{notch} , is the relative importance of A_{tot_del} and represents the effect of notch signaling. We made the assumption that diffusion does not play a role since only the immediately neighboring cells' $delta$ promotes the production of her through notch signaling.

This model simulates the expression of ngn , $delta$, and her through the notch-signaling pathway. The expression of ngn is driven by non-linear autocatalysis and is inhibited by self-saturation and her . The total amount of ngn is equal to the production minus the removal of ngn which is proportional to the number of ngn present. Since the term for basal production of ngn is removed, the initial amount of ngn must be greater than zero. The expression of $delta$ is promoted by ngn but is regulated by self-inhibition. The total amount of delta is calculated by subtracting the amount of delta removed, $R_{del}A_{del}$, from the delta produced. The total her product is linearly proportional to the total delta in the neighboring cells, A_{tot_del} , multiplied by the notch factor, C_{notch} . The following is the algorithm used to simulate the model in NetLogo. Euler's method of integration is implemented with step size of one, $\Delta t = 1$.

Neurogenesis NetLogo Algorithm:

1. Set up the proneuro domains: motorneurons, interneurons, and sensoryneurons. See Fig 4a.
2. Set production (P), removal (R), and saturation and self-inhibition (S) rates. Set notch factor C_{notch} .
3. Set $t = 0$ and $\Delta t = 1$.
4. Set the $A_{ngn}(0)$ of the patches in the domains to user specified value.
5. Optional: Add noise distributed normally with mean and variance set by user, $\alpha A_{ngn}(0) = A_{ngn}(0) + \alpha_1$.
6. If $t = 0$, $A_{del}(0) = P_{del}A_{ngn}(0)$ and if $t > 0$, $A_{del}(t) = A_{del}(t-1) + \Delta t * f_2$.
7. Calculate $A_{tot_del}(t) = \sum_{i=1}^8 A_{del}(t)_i$, where i represents the eight neighboring cells.
8. If $t = 0$, $A_{her}(0) = P_{her}C_{notch}A_{tot_del}(0)$ and if $t > 0$, $A_{her}(t) = A_{her}(t-1) + \Delta t * f_3$.
9. If $A_{ngn}(t)$ is greater than the threshold specified by user, mark the patch as neuron.
10. Set $t = t + 1$.
11. Calculate $A_{ngn}(t) = A_{ngn}(t-1) + \Delta t * f_1$.
12. Optional: Add noise, normally distributed random number with mean = 0 and variance specified by user, $A_{ngn}(t) = A_{ngn}(t) + \alpha_2$
13. Repeat steps 6 – 12.

Somitogenesis

A Lewis model with auto-inhibition and transcription and translation delays was used for the study of somitogenesis [29]. The amount of proteins are calculated as follows,

$$\frac{dp_{her1}}{dt} = a_{her1} [m_{her1}]_{t-T_{mher1}} - b_{her1} [p_{her1}]_t = f_{pher1}, \quad (4)$$

$$\frac{dp_{her7}}{dt} = a_{her7} [m_{her7}]_{t-T_{mher7}} - b_{her7} [p_{her7}]_t = f_{pher7}, \text{ and} \quad (5)$$

$$\frac{dp_{del}}{dt} = a_{del} [m_{del}]_{t-T_{mdel}} - b_{del} [p_{del}]_t = f_{pdel}. \quad (6)$$

The subscripts, *her1*, *her7*, and *del* represent the gene *her1*, *her7*, and *delta*, respectively. The variable p is the amount of protein (indicated by the subscript) at time t . Constants a and b are the rate of protein synthesis and rate of protein degradation, respectively. And, T_{m^*} is the transcription delay of the $*$ protein. The change in the amount of protein is proportional to the amount of mRNA at time $t - T_p$ and minus the amount of protein removed which is proportional to the amount of protein, $b[p]_t$.

The amounts of *her1*, *her7*, and *delta* mRNA are calculated using the following equations:

$$\frac{dm_{her1}}{dt} = [f_{her1}(p_{her1}, p_{her7}, p_{n_del})]_{t-T_{pher1}} - c_{her1} [m_{her1}]_t = f_{mher1}, \quad (7)$$

$$\frac{dm_{her7}}{dt} = [f_{her7}(p_{her1}, p_{her7}, p_{n_del})]_{t-T_{pher7}} - c_{her7} [m_{her7}]_t = f_{mher7}, \text{ and} \quad (8)$$

$$\frac{dm_{del}}{dt} = [f_{del}(p_{her1}, p_{her7}, p_{n_del})]_{t-T_{pdel}} - c_{del} [m_{del}]_t = f_{mdel}. \quad (9)$$

The subscripts are the same as above with an addition of n_delta , which is the total *delta* in the neighboring cells. The constant c is the degradation rate of mRNA. The amount of mRNA production is a function of the amount of *her1*, *her7*, and neighboring *delta* at time $t - T_p$. The variable T_{p^*} is the transcription delay. The mRNA production functions are given below.

$$f_{her1} = k_{her1} \left(r_0 + r_d \frac{\phi_{n_del}}{1 + \phi_{n_del}} + r_h \frac{1}{1 + \phi_{her1} \phi_{her7}} + r_{hd} \frac{\phi_{del}}{(1 + \phi_{del}) (1 + \phi_{her1} \phi_{her7})} \right), \quad (10)$$

$$f_{her7} = k_{her7} \left(r_0 + r_d \frac{\phi_{n_del}}{1 + \phi_{n_del}} + r_h \frac{1}{1 + \phi_{her1} \phi_{her7}} + r_{hd} \frac{\phi_{del}}{(1 + \phi_{del}) (1 + \phi_{her1} \phi_{her7})} \right), \text{ and} \quad (11)$$

$$f_{del} = k_{del} \left(s_0 + s_d \frac{\phi_{n_del}}{1 + \phi_{n_del}} + s_h \frac{1}{1 + \phi_{her1} \phi_{her7}} + s_{dh} \frac{\phi_{del}}{(1 + \phi_{del}) (1 + \phi_{her1} \phi_{her7})} \right). \quad (12)$$

The transcription of *her1*, *her7*, and *delta* of a cell is positively regulated by the neighboring cells' *delta* and negatively regulated by its own *her1* and *her7*. The constant k is the rate of mRNA synthesis. The relative fractional importance on transcription of *her1* and *her7* that is regulated by *delta* alone, by *her1* and *her7* only, and by all three (*delta*, *her1*, and *her7*) are given by r_d , r_h , and r_{dh} , respectively. For *delta* transcription, they are given by s_d , s_h , and s_{dh} . The parameters r_0 and s_0 are for basal or unregulated transcription. The sum of r_0 , r_d , r_h , and r_{dh} and the sum of s_0 , s_d , s_h , and s_{dh} should both equal one. The variable ϕ is the ratio of the current level of protein to the critical protein level when it starts the inhibition of its own production of protein,

$$\phi_{her1} = \frac{P_{her1}}{P_{c_her1}}, \quad (13)$$

$$\phi_{her7} = \frac{P_{her7}}{P_{c_her7}}, \text{ and} \quad (14)$$

$$\phi_{n_del} = \frac{P_{n_del}}{P_{c_del}}. \quad (15)$$

The rate of mRNA degradation is directly proportional to the amount of mRNA. In our analysis, the amount of mRNA at $(t-1)$ is used.

To achieve the wave-like oscillation of *her1* and *her7*, we set up a linear gradient that is a function of the position. In addition, to halt the oscillation and produce spatial patterning, we use an independent moving sigmoidal gradient of *fgf*. In our model, once the *fgf* level falls below the critical *fgf* level and *her1* is above a threshold, the expression of *her1*, *her7*, and *delta* are stabilized at those current levels. The *fgf* level controls when somites begin to form (stabilized gene expression), and the *her1* threshold allows us to skip the small peak in the beginning of the oscillation to avoid peaks that occur prior to the development of full oscillation. To integrate this system of differential equations, we used Euler's method with $\Delta t = 1$. The implementation of this model in NetLogo is described below.

Somitogenesis NetLogo Algorithm:

1. Set the synthesis (a and k), degradation (b and c), and delay (T_m and T_p) constants for mRNA and protein of *her1*, *her7*, and *delta*. Set the critical protein value (p_{crit}), of the three genes.
2. Set r_0 , r_d , r_h , s_0 , s_d , and s_h . Calculate $r_{dh} = 1 - r_0 - r_d - r_h$ and $s_{dh} = 1 - s_0 - s_d - s_h$.
3. Set $t = 0$ and $\Delta t = 1$.
4. Set $p_{her1} = p_{her7} = p_{del} = 0$.
5. Set up the domain. For the patches in the domain do the following steps.
6. Set up the initial linear gradient of *her1* and *her7*, $m_{her} = -C_1x + C_2$, where m is the number of mRNA, C_1 is the slope of the gradient, x is x-coordinate of the patch, and C_2 is the maximum value of *her1/7*.
7. Set up the initial sinusoidal *fgf* gradient, $Fgf = \frac{C_3}{1 + 10^{-(x+(C_4-tM)/C_5)}}$, where C_3 is the maximum *fgf*, C_4 determines the location, C_5 determines the shape of the gradient, and M determines the rate of the gradient movement.
8. Set the rate of domain extension, E .
9. Optional: add noise. The current number of $m_{her1}[0]$ and $m_{her7}[0]$ are replaced by the absolute value of a normal random number generated with mean of current number and variance specified by user.
10. If $t < (T_m + T_p)_{her1}$, set $m_{her1}(t) = m_{her1}(t-1) - c_{her1}m_{her1}(t-1)$.
 If $t < (T_m + T_p)_{her7}$, set $m_{her7}(t) = m_{her7}(t-1) - c_{her7}m_{her7}(t-1)$.
 If $t < (T_m + T_p)_{del}$, set $m_{del}(t) = m_{del}(t-1) - c_{del}m_{del}(t-1)$.

11. If $t > (T_m + T_p)_{her1}$, set $\phi_{her1} = \frac{p_{her1}(t - T_{pher1})}{P_{c_her1}}$ and $m_{her1}(t) = m_{her1}(t-1) + \Delta t * f_{mher1}$.
- If $t > (T_m + T_p)_{her7}$, set $\phi_{her7} = \frac{p_{her7}(t - T_{pher7})}{P_{c_her7}}$ and $m_{her7}(t) = m_{her7}(t-1) + \Delta t * f_{mher7}$.
- If $t > (T_m + T_p)_{del}$, set $\phi_{del} = \frac{p_{del}(t - T_{pdel})}{P_{c_del}}$ and $m_{del}(t) = m_{del}(t-1) + \Delta t * f_{del}$.
12. If $t > T_{mher1}$, set $p_{her1}(t) = p_{her1}(t-1) + \Delta t * f_{pher1}$.
- If $t > T_{mher7}$, set $p_{her7}(t) = p_{her7}(t-1) + \Delta t * f_{pher7}$.
- If $t > T_{mdel}$, set $p_{del}(t) = p_{del}(t-1) + \Delta t * f_{pdel}$.
13. If *fgf* level is less than the critical *fgf* level and $p_{her1} < \text{threshold}$, both set by user, stop m and p calculations.
14. Extend the domain by one each time the rounded value of t/E increased by one.
15. Move *fgf* gradient with the rate specified by the user.
16. Set $t = t + 1$.
17. Repeat steps 10 - 16.

Anterior/Posterior Patterning

The anterior/posterior patterning under the influence of a morphogen gradient simulation is based on Meinhardt's model [27-28]. Meinhardt proposed that for only one gene to maintain primary expression in a cell, it must exhibit autocatalysis and inhibit all alternative genes. In addition, genes should subsequently initiate the expression of another gene in the presence of a morphogen: gene A initiates gene B, which initiates gene C, which initiates gene D.

$$\frac{dG_a}{dt} = \frac{S_a(G_a^2 + M_t M_a)}{G_a^2 + G_b^2 + G_c^2 + G_d^2 + 1} - R_a G_a = f_a, \quad (16)$$

$$\frac{dG_b}{dt} = \frac{S_b(G_b^2 + M_t M_b G_a)}{G_a^2 + G_b^2 + G_c^2 + G_d^2 + 1} - R_b G_b = f_b, \quad (17)$$

$$\frac{dG_c}{dt} = \frac{S_c(G_c^2 + M_t M_c G_b)}{G_a^2 + G_b^2 + G_c^2 + G_d^2 + 1} - R_c G_c = f_c, \text{ and} \quad (18)$$

$$\frac{dG_d}{dt} = \frac{S_d(G_d^2 + M_t M_d G_c)}{G_a^2 + G_b^2 + G_c^2 + G_d^2 + 1} - R_d G_d = f_d. \quad (19)$$

The parameter G is the amount of gene expressed and the subscripts a , b , c , and d represent gene A, gene B, gene C, and gene D. The variables S and R are the rate of synthesis and the rate of removal or degradation, respectively. M_t is the amount of morphogen and M is the fraction of importance the morphogen plays in promoting a particular gene.

Exposure to morphogen is a key factor in determining the pattern of gene expression. It is known that stable, discrete compartments of gene expression can be achieved by activities of a morphogen such as *wnt*. However, the question remains whether the concentration gradient determines gene expression patterns or is this gradient too inefficient and instead time plays the

key role [30]. To study the spatial pattern developed based on a stable and non-moving morphogen, a gradient was set up on a circular domain with the maximum concentration on the edge and minimum in the center. The morphogen level was calculated with the following equation:

$$M_t(x) = \frac{M_{\max}}{C_1 * (x^2 + 1) + M_{\max}} . \quad (20)$$

M_{\max} is the maximum morphogen level, C determines the shape and the steepness of the gradient, and x is the distance from the center of the circle. For our simulation we used $M_{\max} = 1$ and $C = 0.01$.

To examine if a similar pattern is obtainable by the effect of time exposure to morphogen, another circular domain was set up. Initially, the whole region was set to the maximum level of morphogen; as time increases, the morphogen near the center will decay moving outward until all the morphogen levels are zero. The decay is calculated by

$$M_t[t] = M_t[t] - D * M_t[t - 1] , \quad (21)$$

where D is the rate of decay. The time when the decay began is determined by distance from the center of the domain. When $Dist \geq C_2 * t$, where C_2 is the rate of movement and t is the time, the morphogen will begin to decay until the level reaches zero. Again, we used Euler's method of integration with $\Delta t = 1$ for morphogenesis system of equations.

Region 1 (Stable and non-moving morphogen) NetLogo Algorithm:

1. Set $t = 0$, $\Delta t = 1$, and $G_a = G_b = G_c = G_d = 0$.
2. Set synthesis, degradation, and morphogen constants (S and M) to user specified values.
3. Setup the morphogen gradient.
4. Set

$$\begin{aligned} G_a(t) &= G_a(t-1) + \Delta t * f_a , \\ G_b(t) &= G_b(t-1) + \Delta t * f_b , \\ G_c(t) &= G_c(t-1) + \Delta t * f_c , \text{ and} \\ G_d(t) &= G_d(t-1) + \Delta t * f_d . \end{aligned}$$

5. Set $t = t + 1$
6. Repeat step 3 and 4.

Region 2 (Time exposure) NetLogo Algorithm:

1. Set $t = 0$, $\Delta t = 1$, and $G_a = G_b = G_c = G_d = 0$.
2. Set synthesis, degradation, and morphogen constants (S and M) to user specified values.
3. Set M_t to the maximum level.
4. Calculate gene levels, same as step 4 for Region 1.
5. Set $t = t + 1$
6. Recalculate M_t , start decay from center moving toward the edge with each iteration.
7. Repeat step 3 and 5.

Results and Discussion

Neurogenesis

The values used for rates of synthesis, degradation, saturation, self-inhibition, and *ngn* threshold used for the simulation are listed in Table 1. These values were empirically determined. In studying the effect of notch signaling, the number of cells fated to become neurons was determined as a function of C_{notch} (See Table 2). Using our specified values in Table 1 and initial $P_{ngn} = 0.6$, the range of C_{notch} was found to be small, 0 – 0.3, where $C_{notch} = 0$ fated all cells to become neurons and $C_{notch} = 0.3$ fated only 2 cells to become neurons. Since there was no noise in the initial P_{ngn} or in subsequent iterations, the neuron-fated cells are determined solely by the cell's location and the geometry of the domains as illustrated in Fig. 4. This is obvious since cells on the edge of the domains are inhibited less by the neighboring cells.

We chose $C_{notch} = 0.75$ to study the effect the initial amount of *ngn* ($P_{ngn}[0]$), Type 1 noise, and Type 2 noise have on the pattern. The effects of the P_{ngn} initial condition is given in Table 3 and Fig. 5. Upon visual inspection of the pattern with different $P_{ngn}[0]$ and comparing the change in the number of neuron-fated cells, two critical points were observed, $P_{ngn}[0] = 0.53$ and $P_{ngn}[0] = 0.63$. The introduction of Type 1 and Type 2 noise (described below) slightly decreases the overall number of neuron-fated cells, but increases the number of neuron-fated cells in the inner regions of the domains.

The effects of Type 1 and Type 2 noise were tested with $P_{ngn}[0] = 0.58$, since it was the midpoint of the two critical points; results are given in Table 4, Table 5, and Fig. 6. Type 1 noise is variance introduced only to the $P_{ngn}[0]$. This is achieved by replacing the initial P_{ngn} with a random number generated by a normal distribution with mean = P_{ngn} and different variance. Type 2 noise is Type 1 noise plus normally distributed noise (mean = 0 and variance specified by user) between iterations. The variance should be small to represent small differences in gene expression and therefore the variance of 0.02, 0.03, and 0.04 were chosen. From the visual inspection, it can be concluded that Type 1 noise affects the pattern the most, while Type 2 noise has a secondary effect. Of course, increasing the mean of the noise added during iteration will increase the importance of the Type 2 noise.

Somitogenesis

The synthesis and degradation rates, delay, critical protein levels, *her1* threshold, and relative importance of regulation used in our simulation were taken from Lewis's model and are listed in Table 7a & 7b. We use the following equations for *her1/her7* gradients and *fgf* gradient,

$$m_{(her1,her7)} = 1 * x + 18 \text{ and}$$
$$fgf = \frac{50}{1 + 10^{-(x+(80-0.3t))/38.2}}.$$

The variable x is the x-coordinate of the cell. The initial domain and the initial gradients are from $x = -80$ to $x = 0$. The *fgf* gradient moves independently through the domain to the right at the rate of 0.3 per iteration. Simultaneously, the domain also extends at the rate of 0.1 per iteration and takes on the condition of the cell immediately to the left. The critical point for *fgf* is

set at 10. Therefore, the oscillation of genes is halted for patches with *fgf* less than 10 and *her1* greater than 200 (to skip the first peak).

We began by examining the role of the initial *her1/her7* gradients and the initial *fgf* gradients on the formation of somites. The *her1/her7* gradients are found not to play a role in the size or the formation time of somites (Table 8). It does provide the mechanism to produce the sweeping wave-like motion of *her1/her7* expressions across the domain. However, this motion was only sustained for a couple of cycles. To have the motion last longer, it is highly probable that the parameters listed in Table 7 could be tweaked, but this was not examined. Although the shape of the *fgf* gradient is not significant in somite formation time, it does play a role in somite size determination. It can be assumed from Lewis's model that the formation time is highly dependent on the critical protein levels and the transcription and translation delays.

The importance of notch signaling in synchronizing a gene's oscillation of the neighboring cells was examined. Noise was introduced to the initial *her1* and *her7*, determined from the gradient equation, by changing it with a random number generated from a normal distribution with a mean of current *her* and variance ranging from 1 – 5, see Table 9. It shows that when the transcription is highly regulated by delta, *her1*, and *her7* ($r_{dh} = 0.99$), synchronization occurs even with a variance of 5. Although it seems to synchronize at lower r_{dh} (See Fig. 7), it is hard to make any conclusions since oscillation ceases at r_{dh} below 0.98.

Anterior/Posterior Patterning

Similar patterns of gene expressions under the influence of a morphogen can be obtain from either of the following cases:

1. $S_a > S_b > S_c > S_d$ and $M_a < M_b < M_c < M_d$ or
2. $S_a < S_b < S_c < S_d$ and $M_a > M_b > M_c > M_d$.

The values used for the remaining parameters are given in Table 10a and 10b. In both cases, gene A is first expressed, then gene B is promoted except for a small region at the center of the circle. As time increases, in the gene B expressing region, gene C is promoted except for a region around the stable gene A expressing region. Finally, gene D is promoted starting at the edge of the circle (gene C expressing region) and continues to increase until the expressions are all stabilized, see Fig. 8 and Fig. 9.

Furthermore, patterns due to time exposure to morphogen can produce patterns similar to those obtained by a stable exposure to the morphogen gradient by tweaking the decay rate of morphogen and speed of the increase in the decay region of the time-exposure study.

Future Work

First, our study of the systems of equations presented here is very basic. Numerical analyses were performed for all these systems using Euler's method with step size of one. We did not study the error associated with this method. It would be valuable to examine just how much of the result is due to bias in the numerical method and how much is actually due to the biological process model. Perhaps, use the Runge-Kutta method which is known to be a more accurate numerical procedure for obtaining approximate solutions to differential equations. Furthermore,

it would be ideal to perform dynamical systems analysis to better model and understand these systems.

Secondly, our simulation can be modified to be more robust, perhaps by extending the models to incorporate more factors. It is important that enough aspects of the system are taken into account and are accurately known in the models.

For neurogenesis, the model can be modified to include a factor to study the unregulated expression of *ngn*. The noise added during iteration can be construed as the basal expression, but separating the two will allow users to better study and manipulate the model system.

The independent moving gradient in somitogenesis simulation is to halt the oscillation seems to be a brute force way to achieve our objective. It may be more reasonable to incorporate *fgf* in the equations to stop the oscillation, then to reach and maintain *her1*, *her2*, and *delta* expressions.

In the morphogenesis model, two things can be added to improve the model. The first one is a minor one; instead of setting up the regions from the start, allow the region to grow with time. This may give an insight into how cell growth plays a role in the pattern formation. The second one, the effect of the antagonist on the morphogen should be further studied. This has been studied and in the anterior/posterior patterning, *tcf* has been shown to play a critical role in determining the sizes of midbrain and hindbrain. This has been studied for the morphogen gradient case but not thoroughly studied for the time-exposure case.

There have been little or no mathematical-model studies for neurogenesis, somitogenesis, and morphogenesis. Although our study here is basic, overall, modeling is an excellent way to study biological processes. The NetLogo environment provides a world where cell-cell communication alone with gene interaction among itself and the environment can be scrutinized and studied effectively. However, modeling alone is not enough to make conclusions regarding biological systems. It is necessary to use simulation in parallel with bench work to design better experiments and to present compelling evidence for conclusions drawn.

Table 1 These are the values used in the analysis for the rate of synthesis (P) and rate of degradation (R) for *ngn*, *delta*, and *her*. The last column is the rate of saturation for *ngn* and rate of self-inhibition of *delta*. The threshold of 4 was used for *ngn*.

Parameter	Gene		
	Ngn	delta	her
Rate of synthesis (P)	0.5	9.2	0.25
Rate of Degradation (R)	0.20	0.91	0.90
Rate of Saturation or Self-Inhibition (S)	0.06	0.30	None
Threshold	4		

Table 2 Number of cells that become neurons by varying notch factor with $P_{ngn} [0] = 0.6$, Threshold = 4, and no noise in the initial P_{ngn} . The number of iterations required to reach stabilized gene expression is given in the last column.

Notch Factor (C_{notch})	Neuron	
	Number	Percent
0	1171	100 %
0.025	1171	100 %
0.05	785	67%
0.075	568	49%
0.1	432	37%
0.3	2	< 1 %

Table 3 In determining what initial condition ($P_{ngn}[0]$) to use, two critical points were found, * and **. To examine the role of noise, $P_{ngn}[0] = 0.58$ (midpoint between the two critical points) and $C_{notch} = 0.075$ were used. The number of neurons with Type 1 ($P_{ngn}[0]$ is distributed with mean = 0.58 and variance = 0.03) and Type 2 (Type 1 noise and noise distributed normally with mean = 0 and variance = 0.03 is added to $P_{ngn}[t]$ at every iteration). See Table 4 and Table 5 for details. Number of neurons for Type 1 and Type 2 noise is the average of 10 samples.

P_{ngn}	Number of neurons		
	No noise	Type 1 noise (avg)	Type 2 noise (avg)
0.4	0	-	-
0.5	106	-	-
0.51	110	-	-
0.52	208	-	-
0.53*	430	364	393
0.58	536	543	539
0.6	568	-	-
0.62	622	-	-
0.63**	685	671	668
0.7	721	-	-
0.8	942	-	-
0.9	1105	-	-
1	1171	-	-

Table 4 Number of neurons with Type 1 noise. Average and standard deviation are rounded up to next integer.

Conditions	Number of neurons		
	Variance = 0.02	Variance = 0.03	Variance = 0.04
1. $P_{ngn}[0] = 0.58$	563	533	530
2. $C_{notch} = 0.075$	559	544	532
3. Noise in only $P_{ngn}[0]$ with mean = $P_{ngn}[0]$.	554	548	544
	563	549	531
	553	542	535
	562	546	514
4. Iterate until stabilized.	583	534	524
	555	532	543
	560	545	529
	561	557	534
	Average		
561	543	532	
Standard Deviation			
9	8	9	

Table 5 Number of neuron with Type 2 Noise. Average and standard deviation are rounded up to next integer.

Conditions	Number of neurons		
	Variance = 0.02	Variance = 0.03	Variance = 0.04
1. $P_{ngn} [0] = 0.58$	540	545	549
	554	540	548
2. $C_{notch} = 0.075$	548	539	537
	554	552	546
3. Noise in only $P_{ngn} [0]$ with mean = $P_{ngn} [0]$ and variance = 0.03.	533	524	532
	547	525	529
	543	542	524
	527	536	552
4. Add noise with normal distribution (mean = 0) to P_{ngn} during every iterations.	524	551	527
	540	531	537
	Average		
5. $t = 100$.	541	539	539
	Standard Deviation		
	11	10	11

Table 6 Average number of neurons with varying C_{notch} and Type 1 (mean = $P_{ngn}[0]$ and variance = 0.03) and Type 2 (mean = 0 and variance = 0.03) noise conditions. See Table 4 and Table 5 for details. Sample size is 10.

Notch Factor (C_{notch})	Average Number of Neurons		
	No Noise	Type 1 Noise	Type 2 Noise
0.05	785	995	936
0.075	568	543	539
0.1	432	485	478

Table 7 Parameters and their values used in somitogenesis simulation.

a. Synthesis and degradation rates, transcription and translation delays, critical protein level, and threshold of *her1*.

Parameter	Gene		
	<i>her1</i>	<i>her7</i>	<i>delta</i>
Rate of protein synthesis (a)	4.5	5	4.5
Rate of protein degradation (b)	0.23	0.23	0.23
Transcription delay (T_m)	3	2	6
Rate of mRNA synthesis (k)	33	3	33
Rate of mRNA degradation (c)	0.23	0.23	0.23
Translation delay (T_p)	10	6	26
Critical protein level (P_{crit})	5	40	1000
Threshold	200		

b. The values of relative importance of regulated and unregulated transcription of *her1*, *her7*, and *delta*. The subscribes 0 , d , h , and dh represent unregulated, negatively regulated by *delta*, positively regulated by *her1/7*, and regulated both by *delta* and *her1/7*.

Parameter	<i>her1</i> and <i>her7</i>	<i>delta</i>
R_0	-	0.01
r_d	-	0
r_h	-	0
r_{dh}	-	0.99
S_0	0	-
s_d	0	-
s_h	1	-
s_{dh}	0	-

Table 8 The average time required for formation of 1 somite after 7 somites at varying initial *her1/her7* gradients (C_1) and varying rates of *fgf* movement, M .

Slope of the initial <i>her1/her7</i> gradients, C_1 ($C_2 = 18$)	Rate of <i>fgf</i> movement, M ($C_3 = 50, C_4 = 80,$ and $C_5 = 38.2$)	Time (formation of 1 somite)	Size (1 somite, number of rows)
0	0.2	37	8
0	0.3	37	11
0	0.4	37	18
0.5	0.2	37	8
0.5	0.3	37	11
0.5	0.4	37	18
1	0.2	37	8
1	0.3	37	11
1	0.4	37	18

Table 9 The expression levels of p_{her1} for 3 neighboring cells lined up in a column at different important levels of regulated transcription (r_{dh}), variance (var) and time (t).

r_{dh}	Var	Expression of <i>her1</i> (p_{her1}) for Three Neighbor Cells								
		t = 10			t = 30			t = 100		
0.99	1	75.96	76.98	78.10	38.82	38.93	39.06	117.15	116.99	116.95
	2	88.23	89.64	63.47	40.20	40.36	37.41	116.21	115.99	118.08
	3	75.11	74.31	66.46	38.72	38.63	37.75	116.91	117.07	118.03
	4	69.46	80.01	102.67	38.08	39.27	41.83	117.52	115.87	114.96
	5	79.97	63.12	112.84	39.27	37.37	42.97	116.04	117.72	114.26
0.98	5	98.27	96.13	82.99	71.58	71.34	69.86	78.92	79.03	78.99

Table 10 Values used in the morphogenesis simulation.

a. Parameters where $S_a > S_b > S_c > S_d$ and $M_a < M_b < M_c < M_d$.

Parameter	Gene			
	A	B	C	D
Rate synthesis (S)	2.0	1.9	1.8	1.7
Rate gradation (R)	0.10	0.10	0.10	0.10
Importance of morphogen (M)	0.50	1.75	3.25	4.5
Morphogen	Region 1		Region 2	
Max Morphogen (M_{max})	1.00		1.00	
C_1	0.01		0.01	
Decay Rate (D)	-		0.95	
C_2	-		0.40	

b. Parameters where $S_a < S_b < S_c < S_d$ and $M_a > M_b > M_c > M_d$.

Parameter	Gene			
	A	B	C	D
Rate synthesis (S)	1.8	2.8	4.4	6.7
Rate gradation (R)	0.10	0.10	0.10	0.10
Importance of morphogen (M)	1.10	1.20	1.30	1.40
Morphogen	Region 1		Region 2	
Max Morphogen (M_{max})	1.00		1.00	
C_1	0.01		0.01	
Decay Rate (D)	-		0.30	
C_2	-		1.00	

Figure 1 Lateral inhibition through notch signaling.

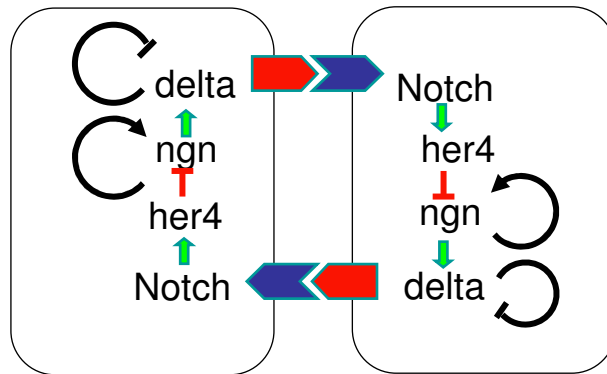


Figure 2 Diagram of the notch signaling pathway for somitogenesis.

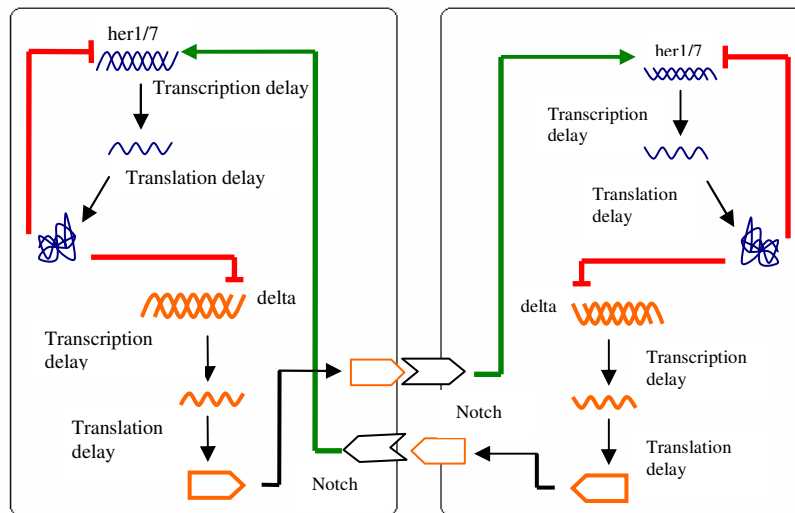


Figure 3 Schematic used in the simulation of spatially differentiated genes under the influence of morphogen.

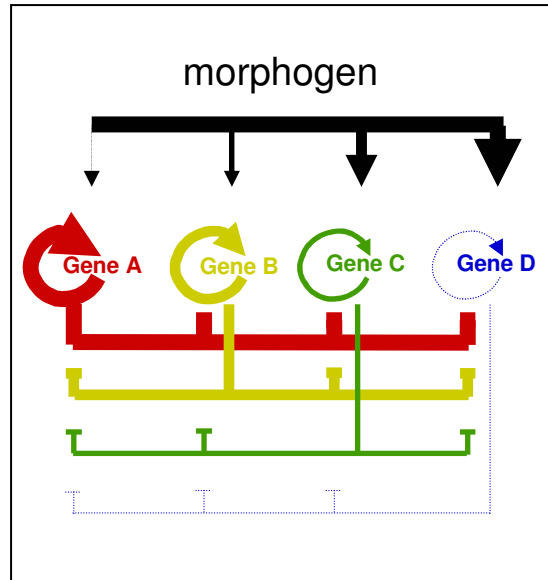


Figure 4 Distributions of cells that are fated to become neurons at different notch factors, C_{notch} , with parameters listed in Table 1, $Pngn[0] = 0.6$, and no noise. At $C_{notch} = 0.025$, all the cells are fated to become neurons.

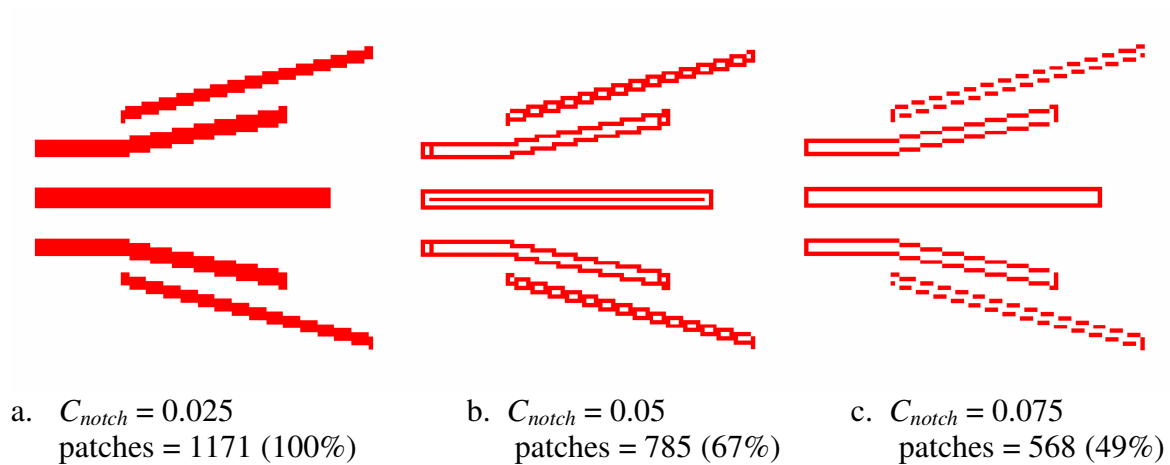


Figure 5 Distributions of cells that are fated to become neurons with different initial ngn levels, $P_{ngn}[0]$ and no noise. The parameters listed in Table 1 were used, along with $C_{notch} = 0.75$. Under these conditions, $P_{ngn}[0] = 0.53$ and $P_{ngn}[0] = 0.63$ are two critical points.

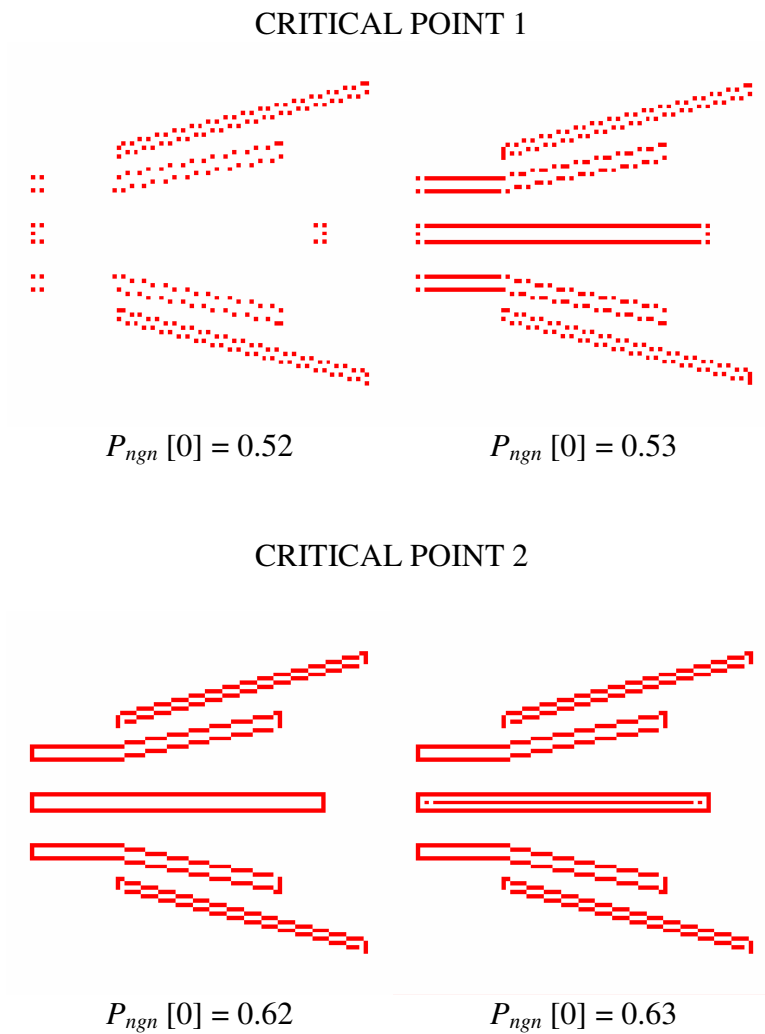
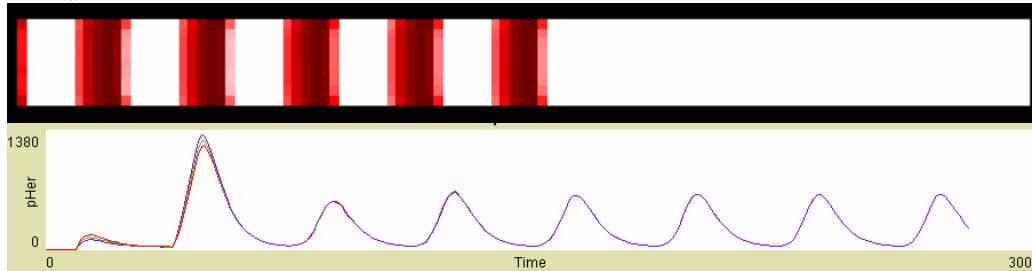


Figure 6 Effects of Type 1 noise where $P_{ngn}[0]$ is a normal distribution with mean = 0.58 and different variance (var). Parameters in Table 1 and $C_{notch} = 0.075$ were used. Effects of Type 2 noise where $P_{ngn}[0]$ is a normal distribution with mean = 0.58 and var = 0.03 and noise that is normally distributed with mean = 0 and different variance was added to $P_{ngn}[t]$ at each interval. Parameters in Table 1 and $C_{notch} = 0.075$ were used.

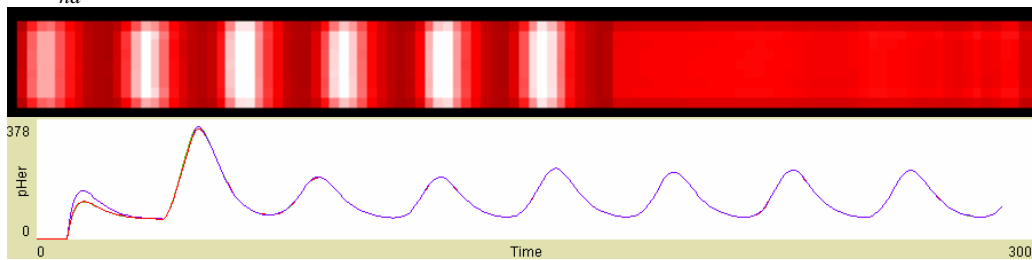


Figure 7 Somites formation simulation using NetLogo. The upper pictures represent the presomitic mesoderm region budding off somites represented by expression of *her1*, white is low expression and red is high expression. The bottom graphs shows the synchronization of *her1* in 3 neighboring patches located in a column for different r_{hd} values.

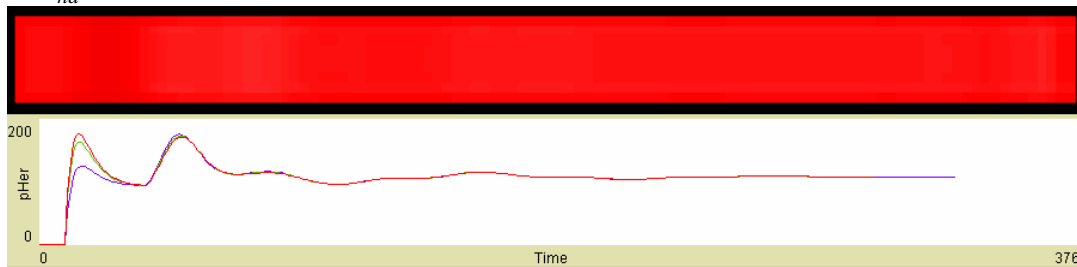
a. $r_{hd} = 0.99$



b. $r_{hd} = 0.98$



c. $r_{hd} = 0.97$



d. $r_{hd} = 0.70$

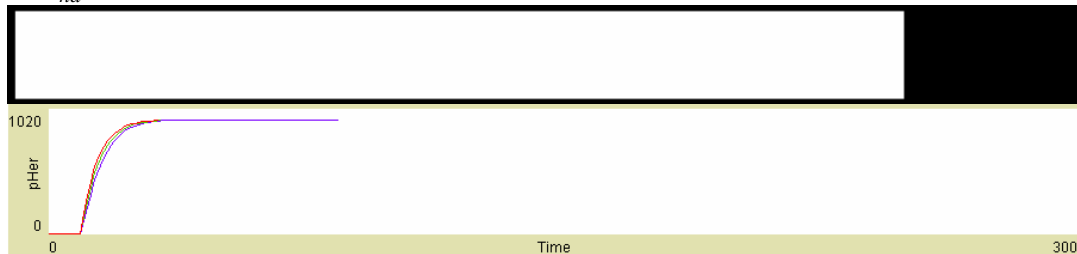


Figure 8 Graphical representation of the gene expression for different regions using $S_a < S_b < S_c < S_d$ and $M_a > M_b > M_c > M_d$, and $R_a = R_b = R_c = R_d$ (see Table 10b for values). a. Region where gene A is expressed, b. Region where B is expressed, c. Region where C is expressed, and d. Region where D is expressed. Overtime the expression of these genes are stable and are able to inhibit the expression of alternate genes within a specific region.

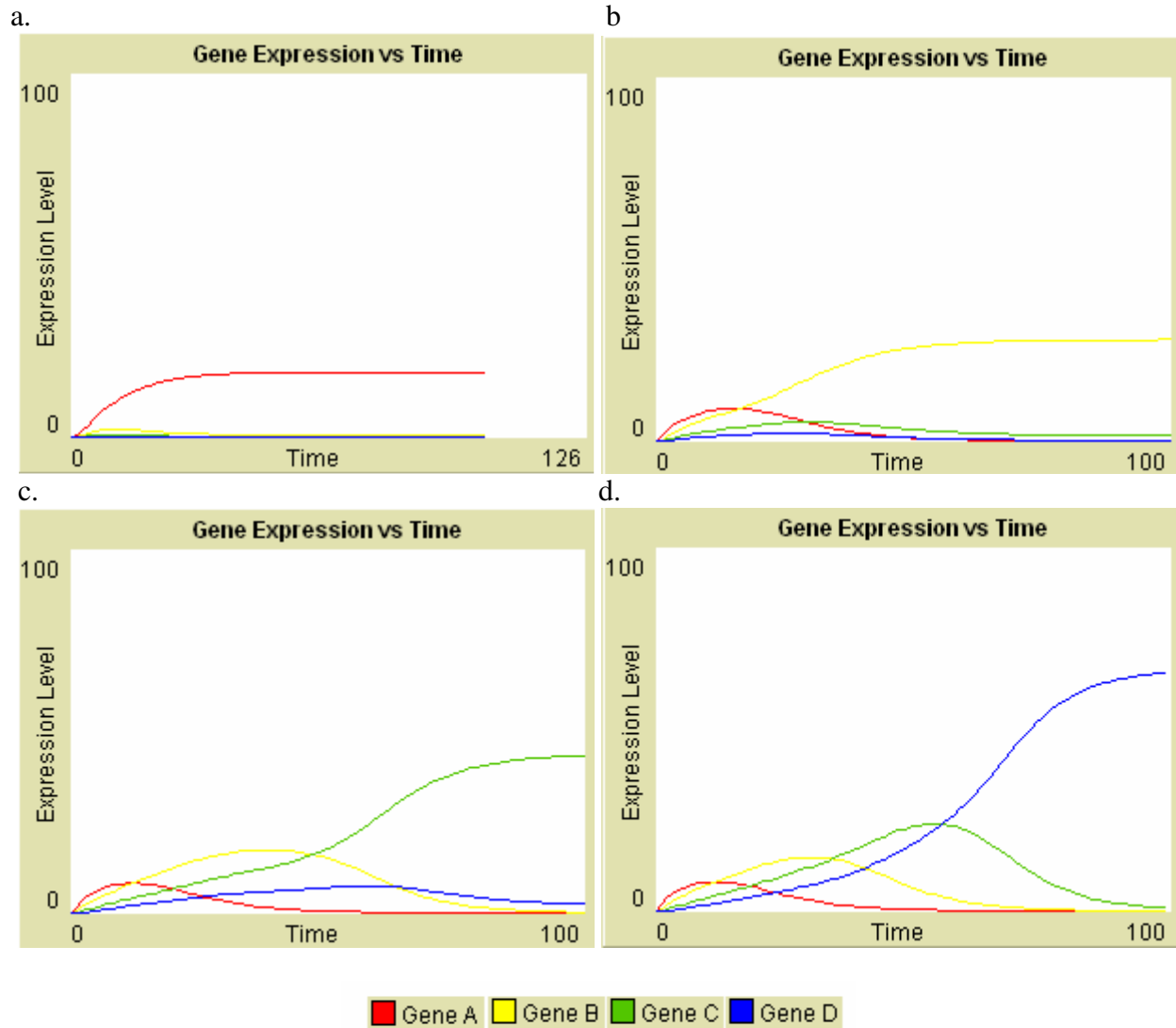
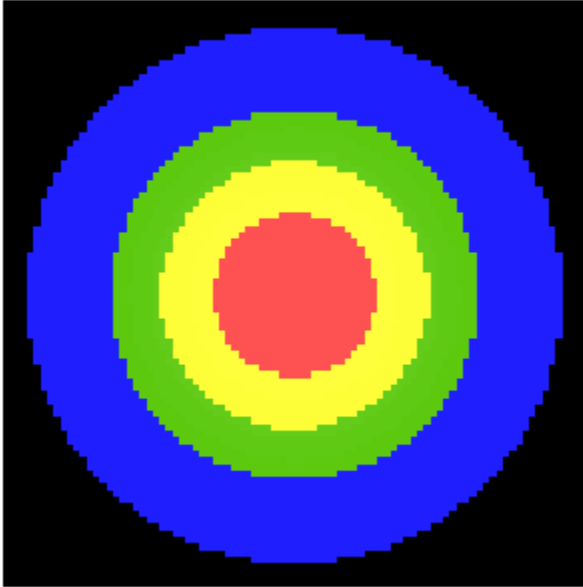


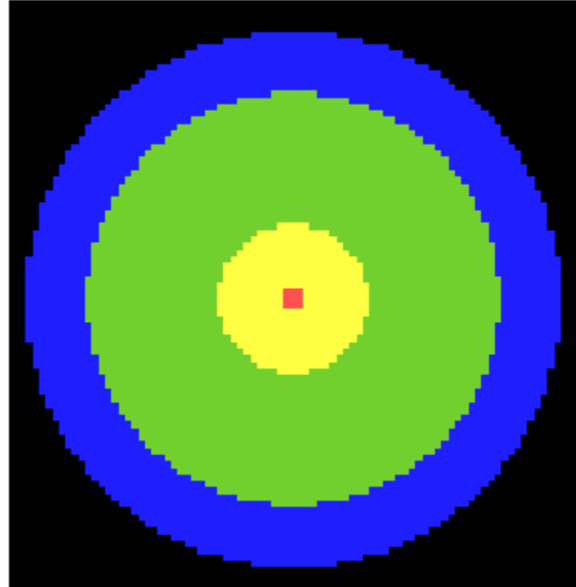
Figure 9 Pattern formation in gene expression under the influence of a morphogen. a. and b. are patterns obtained using parameter values from Table 10a, and c. and d. are patterns using parameter values from Table 10b.

a.



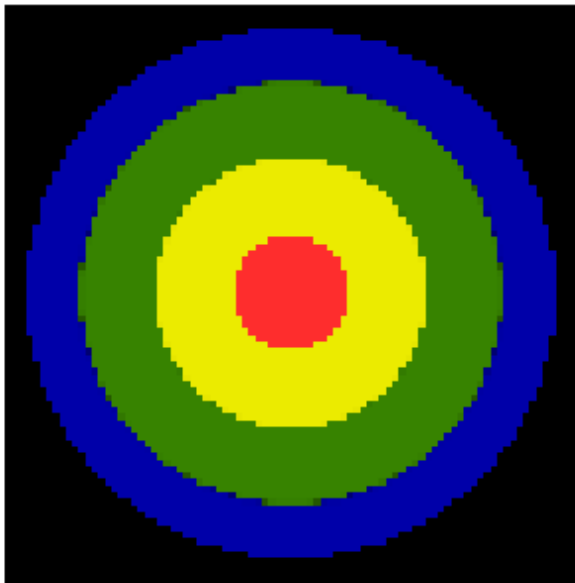
Stable-Morphogen Concentration Study

b.



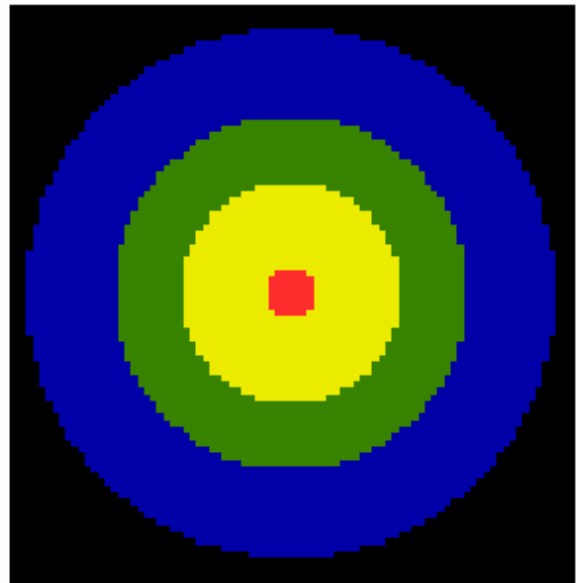
Morphogen Time-Exposure Study

c.



Stable-Morphogen Concentration Study

d.



Morphogen Time-Exposure Study

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